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A new species of *Neocarus* (Opilioacaridae) from a Brazilian ferruginous geosystem and notes on natural history

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Abstract

A new species of *Neocarus* is described from adult female and male specimens collected from an epigean and hypogean ferruginous geosystem located in southeast Brazil. The new species, *Neocarus simmonsi* **sp. nov.**, possesses 15–17 *ch*-type palp setae, nude female pregenital region, cylindrical ovipositor without setae, and a peculiar variation of setae in the genital and pregenital region of the male, with smooth and tapering setae and/or barbed and tapering setae and/or stout and ribbed setae. Female genital setae are barbed, tapering and with a thin tip. Aspects of the ecology and life history of these mites are also presented.

Keywords

Acari, Biodiversity, Cave, Opilioacarida, Parasitiformes, South America

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Introduction

The order Opilioacarida constitutes a cosmopolitan group whose distribution encompasses 26 countries (United States, Mexico, Belize, Cuba, Puerto Rico, Nicaragua, Costa Rica, Panama, Venezuela, Brazil, Argentina, Uruguay, Italy, Greece, Algeria, Angola, Gabon, Madagascar, Ivory Coast, Tanzania, South Africa, Yemen, Kazakhstan, India, Thailand and Australia) and all continents except Antarctica (e.g. Coineau and van der Hammen 1974; Juvara-Bals and Baltac 1977; Walter and Proctor 1998; Thaler and Knoflach 2002; Das and Bastawade 2006; Vázquez and Klompen 2009; Vázquez and Klompen 2010; Bernardi et al. 2012; Vázquez et al. 2014; Vázquez and Klompen 2015; Vázquez and Klompen 2018). The Americas stand out as a region of great diversity with 26 described species and four genera. Brazil presents the greatest diversity known to date, with 11 species and four genera, of which *Brasilacarus* and *Amazonacarus* are endemic to the country, while *Neocarus* and *Caribeacarus* also occur in other regions (van der Hammen 1969; Vázquez and Klompen 2009; Bernardi et al. 2013b, c; Vázquez et al. 2015; Araújo et al. 2018a; Bernardi and Borges-Filho 2018; Vázquez et al. 2020). Nonetheless, the number of known species is likely to represent only a small fraction of the group's diversity, as new records of undescribed Opilioacarida have been reported (Bernardi and Borges-Filho 2018; Monte and Bichuette 2020).

Opilioacarida are edaphic mites found in a wide breadth of habitats, such as soils of forests or dry areas, in the midst of litter, under rocks or tree trunks, and in caves. Several of the species in Brazil have been collected in caves, in spite of not presenting morphological specializations for subterranean environments. However, it is still unclear whether these species have a preference for this type of environment or whether these records are due to the increased sampling effort directed to cave fauna in recent years (Bernardi et al. 2012, 2013b, 2013c, 2014; Araújo et al. 2018a; Bernardi and Borges-Filho 2018). Collections of Opilioacarida in inventories of cave communities have generally been sporadic, with little information being recorded about the biology of these organisms. Such collections in general, result from two types of researches; ecological or faunistic studies focused on describing cave invertebrate communities (e.g. Simões et al. 2015; Monte and Bichuette 2020), and legal obligations in compliance with the environmental licensing of infrastructure projects (e.g. mining, hydroelectric plants, road construction), with the main objective of estimating species richness in the environment of interest (MMA 2009). These observations of specimens of Opilioacarida in Brazilian caves have raised questions about the importance of this type of environment in the life cycle of such organisms, especially considering that some species are abundant in cave environments throughout the year (Bernardi et al. 2013b, 2013c, 2014; Araújo et al. 2018a). The present study describes a new species of *Neocarus* observed in caves and epigean environments in a ferruginous geosystem in Southeast Brazil. In addition, we elucidate some aspects of the biology of this new species of Opilioacarida, such as feeding habits (stomach contents) and seasonality in the subterranean environment.

Material and methods

Morphological study and gut content analysis

Specimens were collected during inventories of cave fauna, which involved thoroughly investigating caves for invertebrates under blocks, in accumulations of organic matter, and in fissures in the soil or cave walls (Wynne et al. 2019). Additionally, a few occasional collections of specimens in epigean environment were performed. Specimens were collected with brushes and stored in containers with 100% alcohol.

Most of the material was studied as slide-mounted specimens. For this purpose, specimens were dissected, cleared in lactic acid and mounted on slides using Hoyer's medium (Walter and Krantz 2009). Stomach contents were extracted from some specimens (n = 10) using micro-needles through a cut in abdomen. Fecal pellets were removed and mounted on microscope slides using hoyer. Some specimens (n = 12) did not require the removal of the stomach contents since clearing of the specimens was sufficient for analysis of the food items present in the fecal pellets.

For the terminology for the palp tarsal sensilla we followed Grandjean (1936) as modified by Vázquez and Klompen (2002), for the sternitogenital region we followed Klompen et al. (2015), and for the leg sensory structures Grandjean (1936), van der Hammen (1966), and Araújo et al. (2018b). Morphological characters were processed in the data matrix development mode of vSysLab (Johnson 2010) and were exported as proto-species descriptions.

Drawings were prepared using a Leica MDLS phase contrast microscope (Leica Microsystens, Wetzlar, Germany), connected to a drawing tube. Measurements were taken from adults using an ocular micrometer and are presented in micrometers (μm), average length is presented first, followed by length range in parentheses. Photos were taken with a 3.2 mega-pixel digital camera attached directly to a microscope.

Collection sites of the specimens examined were georeferenced using coordinates in degrees, minutes and seconds with the World Geodesic System (Datum WGS84).

All specimens are deposited in the following collections:

MZLQ Coleção de Referência Acarologica, Universidade de São Paulo, Escola Superior de Agricultura 'Luiz de Queiroz', Departamento de Entomologia e Acarologia, Piracicaba, São Paulo, Brazil;

ISLA Coleção de Invertebrados Subterrâneos de Lavras, Universidade Federal de Lavras, Departamento de Biologia, Setor de Zoologia, Lavras, Minas Gerais, Brazil;

UFMG AC Acarological Collection at Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Belo Horizonte, Minas Gerais, Brazil.

Abbreviations: **F** = female, **M** = male.

Biological remarks

Observations on seasonality were obtained from data produced by inventories of cave fauna in the municipalities of São Gonçalo do Rio Abaixo and Barão de Cocais, state of Minas Gerais, which used the method of active search as described above. The study was carried out in 109 cavities, with rainy season collections being made in January 2015 and dry season collections in October 2016.

Generalized linear models (GLMs), with contrast analysis were used to determine if there was a significant difference in the size of the *Neocarus* populations in the two seasonal campaigns. The models were built using abundance in each of the caves where *Neocarus* was observed as the response variable and each campaign as the explanatory variable. A negative binomial distribution with a log link functions was used since data presented significant overdispersion for Poisson error distribution (3.860). The GLM regression analysis was performed with R software (R Development Core Team 2019).

Taxonomic section

All specimens examined in this study are assigned to the genus *Neocarus* Chamberlin and Mulaik. Generic assignment is based on the following characteristics:

1. adults with 3 ribbed and stout setae on the penultimate body segment;
2. 4–6 foliate setae on the palp tarsus with no more than 5 lobes each;
3. pectinate (*d2*) setae on palp absent;
4. eupathidium (ζ1) in the main sensillar group of tarsus I and not crown like;
5. shiny fleshy setae with a whip-like tip absent from the palps;
6. dorsal segments VII to XVI and ventral segments X to XVI of the body without setae (according to Klompen et al. 2015).

Arachnida Lamarck, 1802
Parasitiformes Reuter, 1909
Opilioacaridae With, 1902
***Neocarus* Chamberlin & Mulaik, 1942**

***Neocarus simmonsi* sp. nov.**
http://zoobank.org/6DFAE428-71BE-46DF-B6C2-F9F124A27428

Diagnosis. Palp genu without *p*-type setae, tarsus with 15–17 *ch*-type sensilla and typical 6 pairs of foliate setae each with 1 small and thin lobe, plus 3 larger lobes with rounded, not filiform, tips. Sexual dimorphism in setation of prodorsal shield absent. Sternal setae *St2* and *St3* with attenuate tips. Pregenital area in female nude, and genital area with 6–12 barbed, tapering setae with thin tip. Pregenital areas in male with

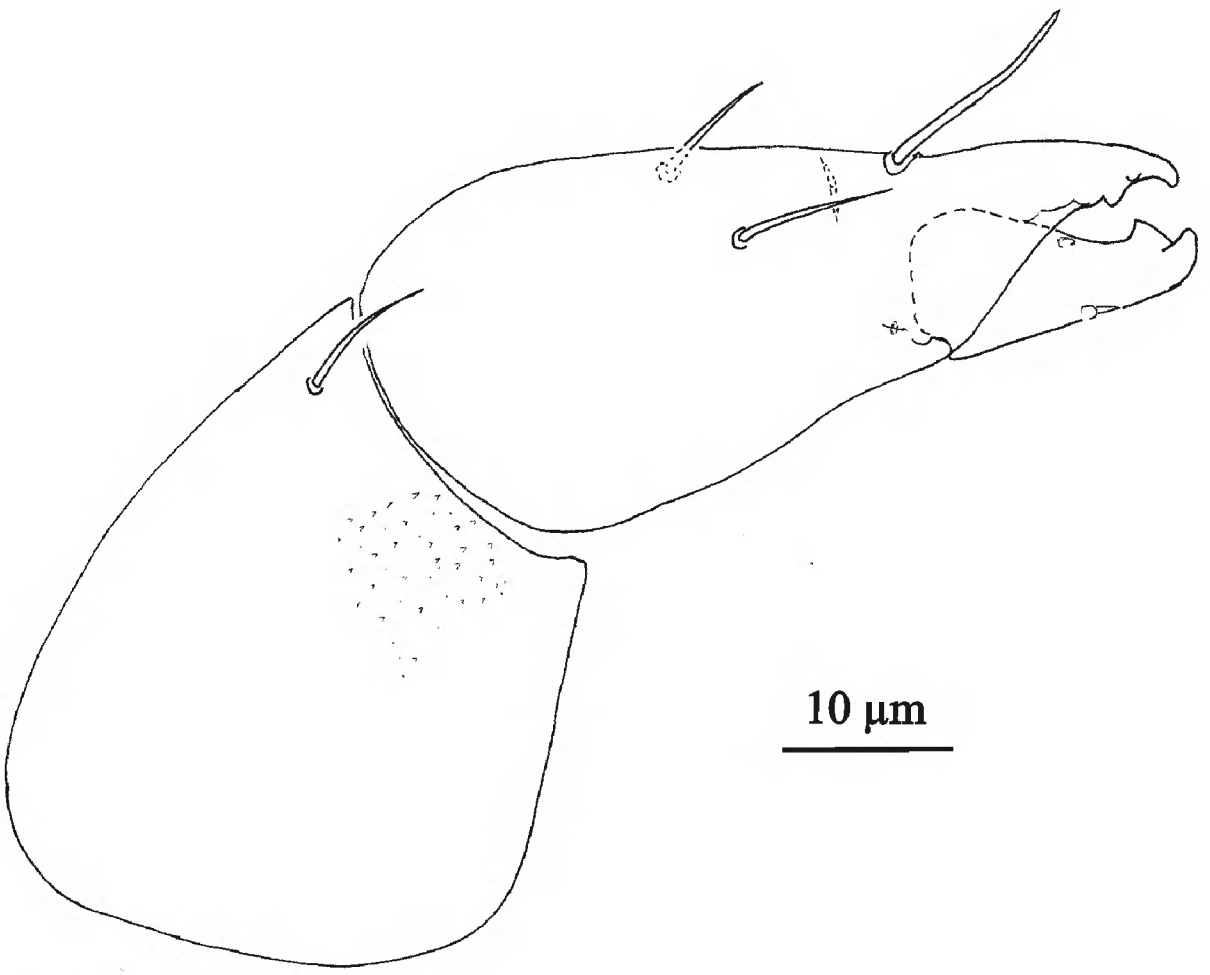

Figure 1. *Neocarus simmonsi* sp. nov., chelicera.

1–6 stout, ribbed, and relatively blunt-tipped setae and 3–7 smooth/light barbed and tapering setae; genital area with 0–6 stout, ribbed, relatively blunt-tipped setae and 4–7 smooth/light barbed and tapering setae. Ovipositor nude, simple tube-like structure, without terminal setiform sensilla or lobes.

Description. Based on 13 females and 6 males.

Gnathosoma. *Chelicera* (Fig. 1). Basal segment in adults with 1 seta, fixed digit with 3, one of which (*ch1"*) distinctly larger. Seta *cht* on basal segment of male chelicera shorter than seta *ch1"* on fixed digit. Setae *ch2'*, *ch2"* and *cht* simple, with an attenuated tip. Movable digit with one ventral denticle in a basal portion. Axial scale-like processes on movable digit in both adults absent. Basal segment 166 (136–190 µm), fixed digit 231 (217–259 µm) and movable digit 79 (74–83 µm).

Subcapitulum (Figs 2, 3). All four pairs of paralabial setae present: *pl1* relatively large, conical, With's organ (*pl2*) membranous and discoid with a biramous core; rutellum (*pl3*) with one row of 5 teeth, inserted dorso-lateral; *pl4* small, inserted dorsal. With 4 circumbuccal (*cb*) and 8–13 median and subcapitular setae (*vm*, *lvm*, *ldm*, *vp*, *lvp*). Seta *vm1* on the male subcapitulum present. Lateral lips with distinct canals (*ogl1* and *ogl2*). Sexual dimorphism evident; in female some circumbuccal setae with a rounded tip (*vm1* and/or *lm1* or/and *vp1*).

Palp (Figs 4, 5, 7D). Trochanter with 0–1 papilliform (= *p*-type) and 3–4 ribbed, tapering setae (= *r*-type); femur with 9–11 papilliform (= *p*-type) and 6–8 *r*-type setae; genu with 2–7 *p*-type and 20–28 *r*-type setae; tibia with 21 long, lightly serrate and pointed setae, 38 *r*-type setae, and 2 long, thin, and smooth sensilla. Tibia and genu ventrally with

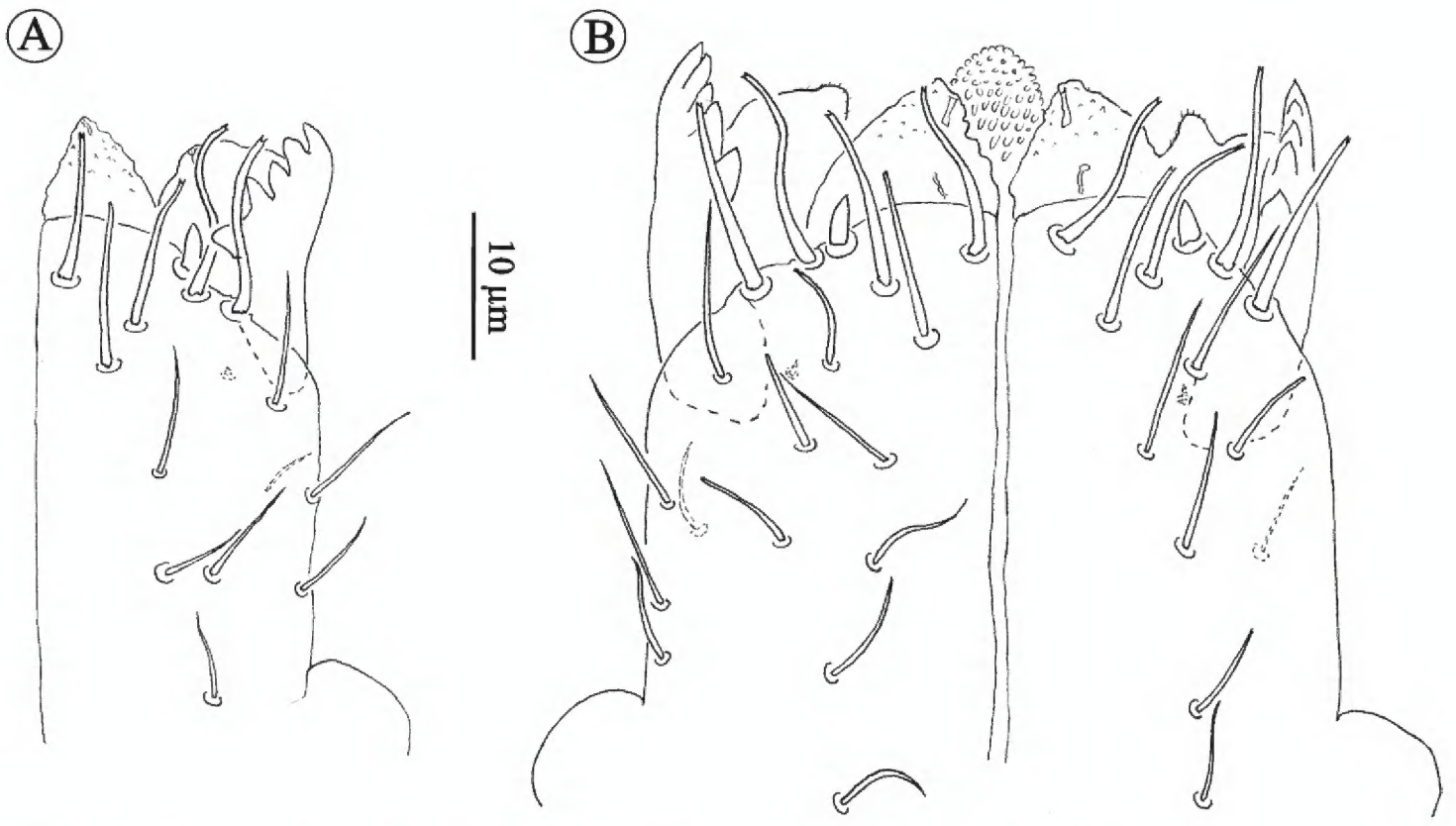

Figure 2. *Neocarus simmonsi* sp. nov., details of subcapitulum **A** male **B** female.

a small indistinct gland. Tibia and tarsus partially fused. Tarsus with 5–6 foliate (*d*-type), 10 *v*, 20 *ch*, and 10–12 *sm* sensilla, plus 3 solenidia (= *s*-type sensilla). Foliate setae each with 1 small, thin plus 3 larger lobes with rounded, not filiform, tips. Modified *sm3*-type sensilla on male palp tarsus absent. Lyrifissures *iπ* and *ia* distinct. Pretarsus in shape of a pair of well-developed sessile claws. Total length of palp 638 (606–733 µm).

Idiosoma. *Color*: Violet-blue with the usual banding pattern. Color observed for live and alcohol preserved specimens (Fig. 6). Total length of idiosoma 1827–1926 µm.

Dorsum. Prodorsal shield with two pairs of lateral eyes. One pair of prodorsal lyrifissures present. Chaetotaxy in females and males consisting of, respectively, 186–204 and 218–242 setae. Sexual dimorphism in anterior portion of prodorsal shield absent. Dorsal idiosoma between the prodorsal shield and the preanal segment without setae, but with numerous lyrifissures arranged in transverse rows. Setation preanal segment limited to 1 dorsal, and 2 ventro-lateral setae. Anal valves with 7–12 stout, ribbed setae (9–12 in females; 7–9 in males).

Sternitogenital region (Figs 7–9). Sternal verrucae in adults each with 3 large pointed, and 1 composite (*St1*) setae. Setae *St1* subequal in size to *St5*. Setae *St2* and *St3* in females and males barbed, tapering to a fine tip. Remaining sternal region with 4–6 pairs of stout, ribbed setae with blunt tips. Pregenital capsules each with 1 long, tapering seta (*St5*) and 6–8 (female) or 5–6 (male) stout, ribbed setae. Pregenital and genital areas in female with 0–1 ribbed, stout setae (1 seta condition rare, observed in only 2 individuals), and genital area with 6–12 barbed, tapering setae with thin tip. Pregenital areas in male with 1–6 stout, ribbed, and relatively blunt-tipped setae and 3–7 smooth/light barbed and tapering setae; genital area with 0–6 stout, ribbed, and relatively blunt-tipped setae and 4–7 smooth/light barbed and tapering setae. Ovipositor simple tube-like structure, without terminal setiform sensilla. Male genital valves rectangular or curved, not triangular.

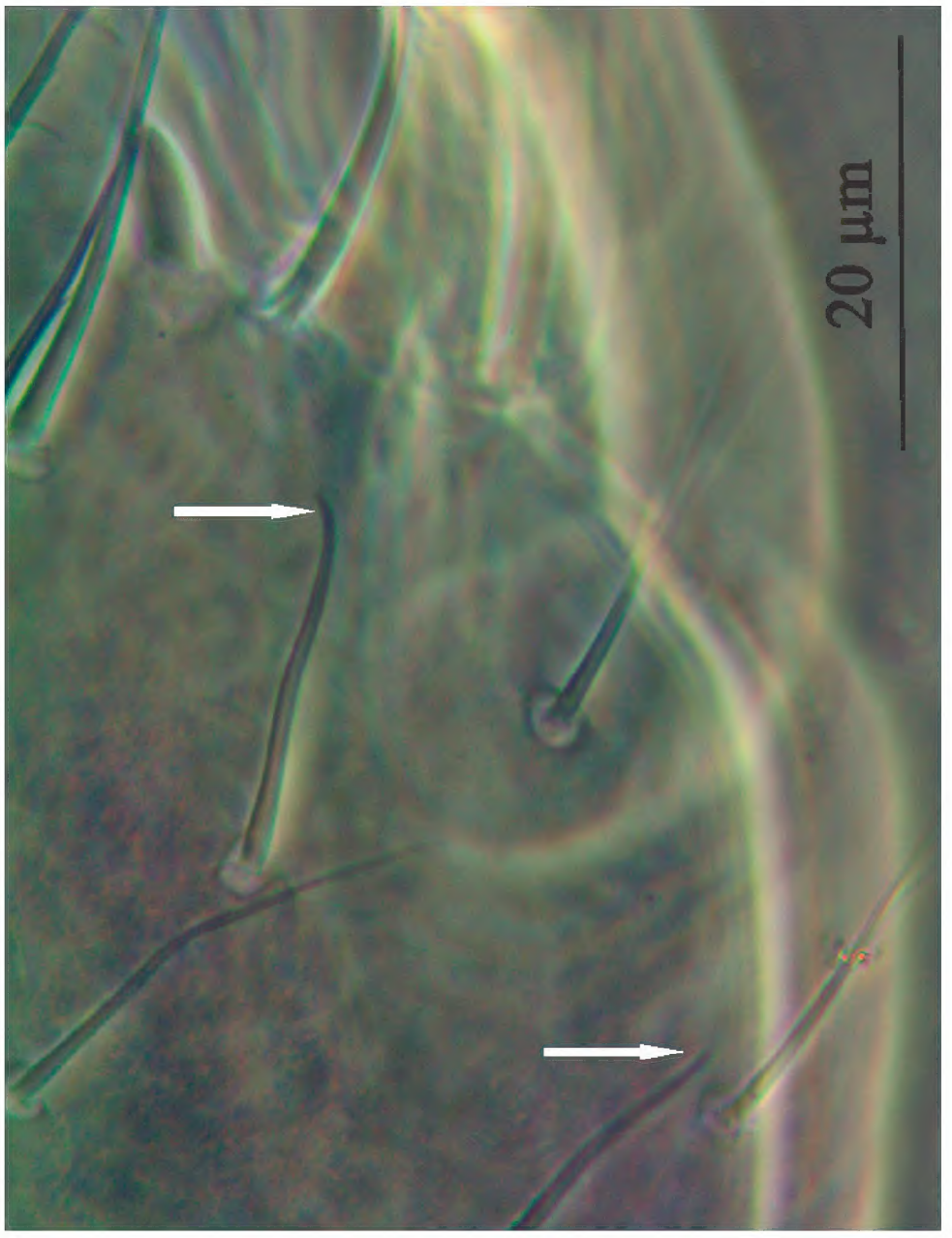

Figure 3. *Neocarus simmonsi* sp. nov., detail of setae with a rounded tip on female (arrowed).

Legs. Length of legs I–IV in females and males very similar, combined as "adults", 3884 (3619–3941), 1973 (1812–2411), 1906 (1652–2047) and 3065 (2741–3520). Eupathidium *z1* on tarsus I inserted in dorsal sensory field; simple, without enlarged tip. Solenidion w*a* on legs II positioned on acrotarsus; w*a* on tarsi III–IV absent. Solenidion w*d* on basitarsi II–IV inserted apically, not sunk into the segment. Ambulacra II–IV in adults with 2 smooth and attenuate setae (*d* and *l*). Ventral portion of acrotarsi II–IV with 3 pairs of setae; lateral portion with 2 pairs distinctly lateral, plus 1 pair of ventro-lateral and one pair of dorso-lateral setae. Setae *lv* of acrotarsi II–IV with one small barb. Papilliform setae on dorsal portion of the basitarsi II–III present. On leg I in both sex, thin and smooth setae restrict to telotarsus and distal portion of basitarsus. Coronidia present on basitarsi II–IV of all adults (basitarsus II 8–14; III 10–16, IV 13–19), absent on tibiae and genua II–IV.

Material examined. Type depository: ***Holotype*** female, UFMG AC: Brazil, Minas Gerais State, São Gonçalo do Rio Abaixo city, Simmons' cave, 19°53'55"S, 43°28'22"W Datum WGS84, 10 Oct 2016, Oliveira MP & Bernardi LFO.

Paratypes: 2 female and 1 male specimens deposited at MZLQ, Cave PDI_0034, 19°53'48.99"S, 43°28'15.29"W Datum WGS84, col Oliveira MP, 06

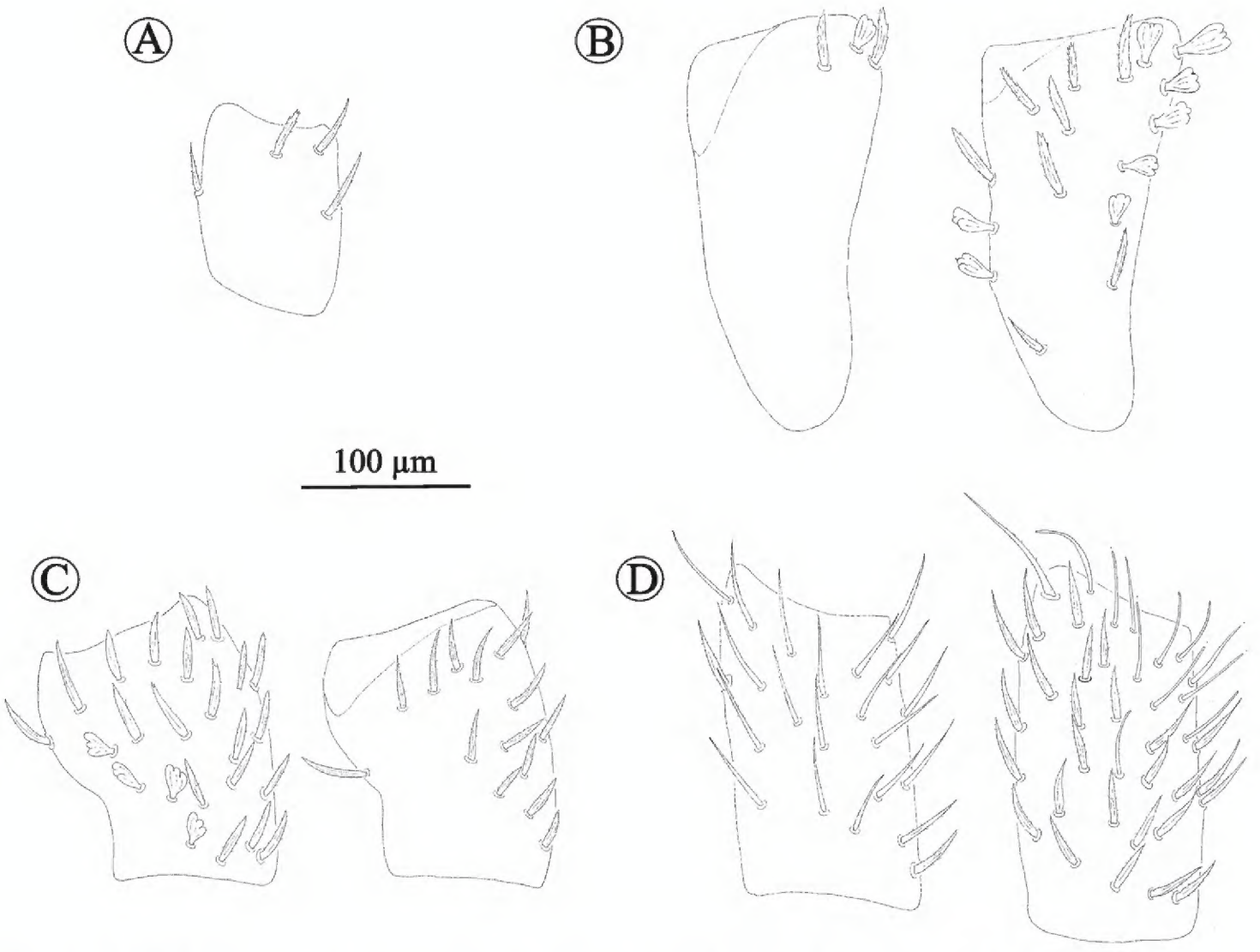

Figure 4. *Neocarus simmonsi* sp. nov., detail of setae on male palp **A** trochanter **B** femur **C** genu **D** tibia.

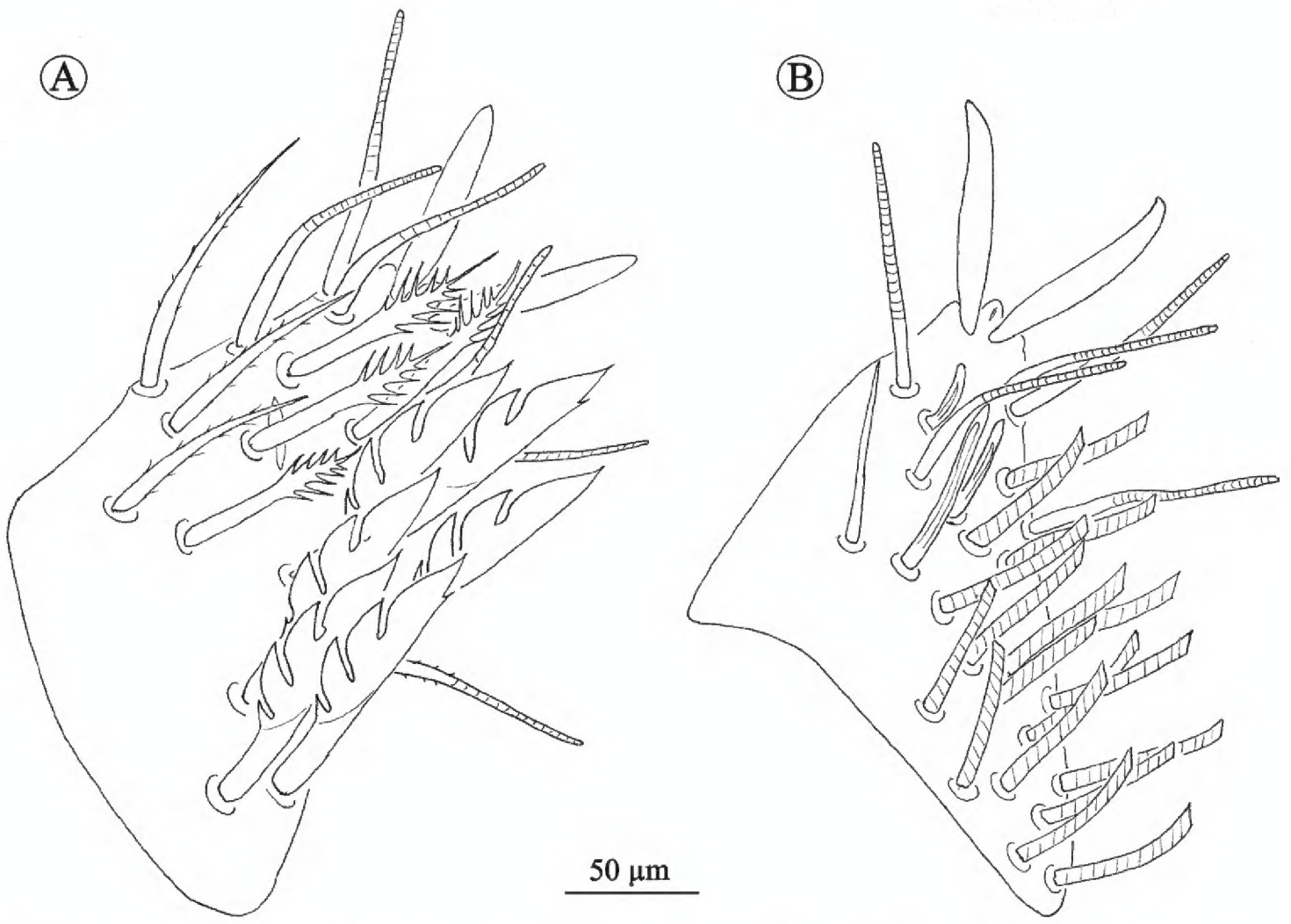

Figure 5. *Neocarus simmonsi* sp. nov., detail of setae on male palp tarsus, dorsal (**A**) and ventral (**B**).

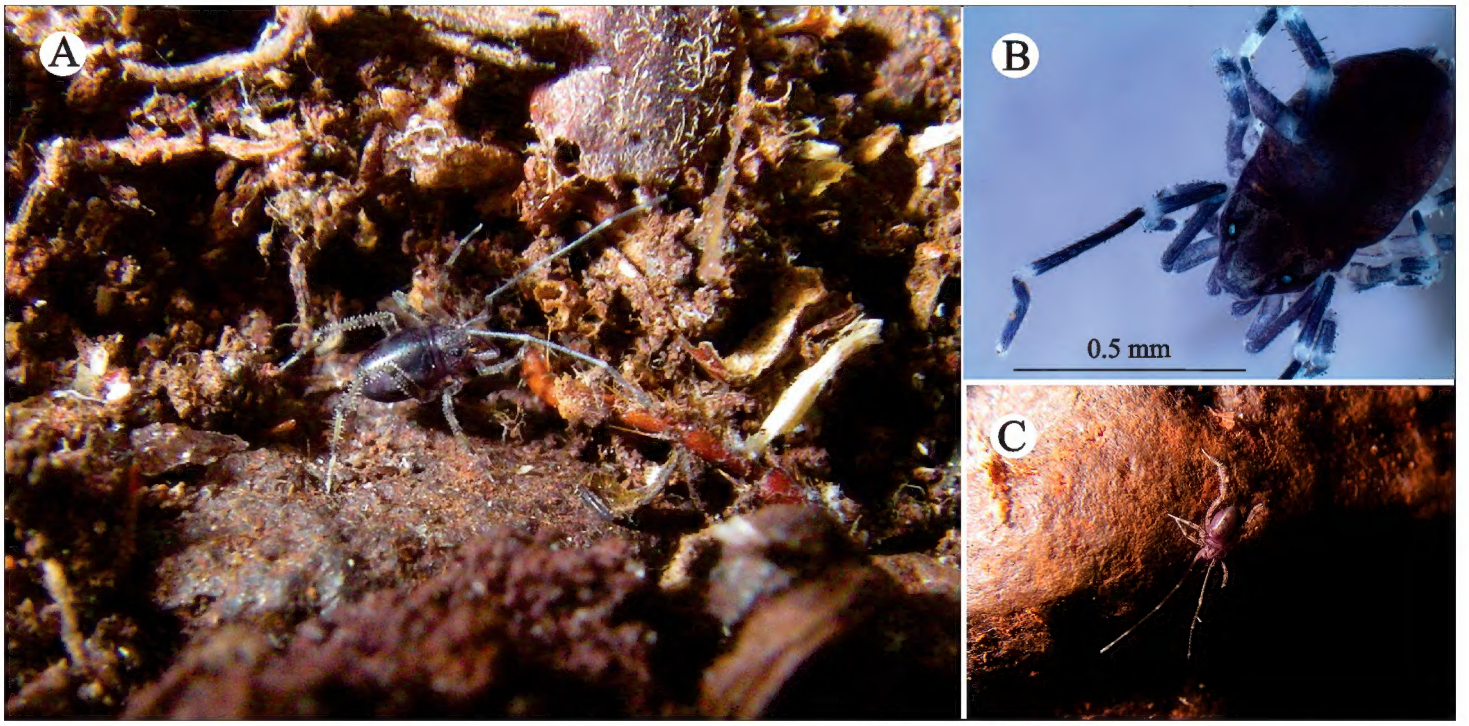

Figure 6. *Neocarus simmonsi* sp. nov., specimens alive observed on cave soil (**A, C**), view of male prodorsal region (**B**).

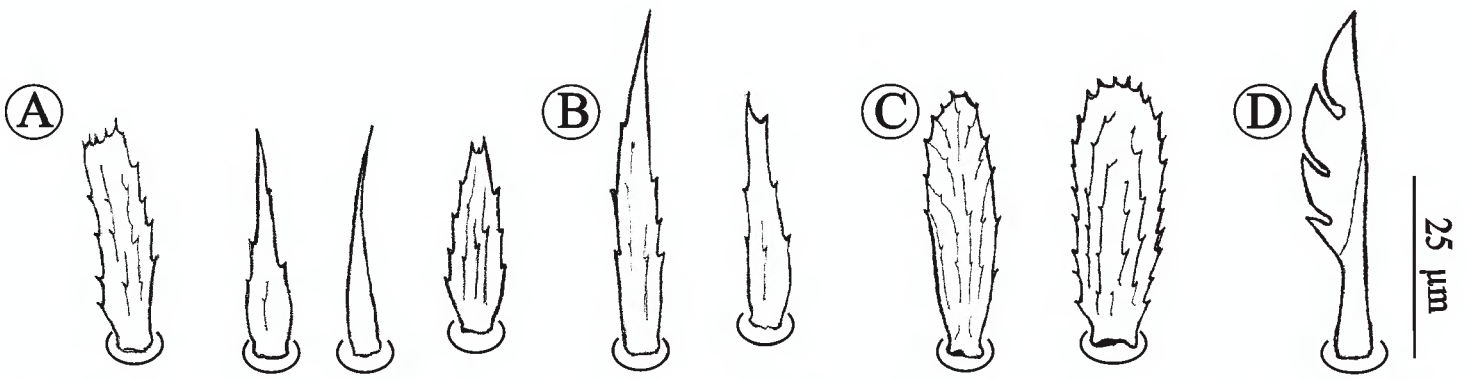

Figure 7. *Neocarus simmonsi* sp. nov., details of setae. **A** Variation on shape of pregenital and gential setae on male **B** variation on shape of gential setae on female **C** detail of setae on prodorsal region **D** detail of *d*-type (foliate seta) on palp tarsus.

Oct 2016; 1 female specimen deposited at MZLQ, cave PDI_0029, 19°53'29.61"S, 43°28'19.74"W Datum WGS84, col Oliveira MP, 26 Nov 2015; 1 female specimen deposited at MZLQ, cave PDI_0059, 19°54'0.75"S, 43°28'9.91"W Datum WGS84, col Oliveira MP, 09 Jan 2016; 1 male specimen deposited at ISLA, cave PDI_0039, 19°53'51.37"S, 43°28'12.51"W Datum WGS84, col Oliveira MP, 05 Oct 2016; 2 females and 1 male specimens deposited at ISLA, cave PDI_0024, 19°53'38.98"S, 43°28'39.14"W Datum WGS84, col Oliveira MP, 26 Nov 2015; 1 female specimen deposited at ISLA, cave PDI_0031, 19°53'49.62"S, 44°28'13.36"W Datum WGS84, col Oliveira MP, 06 Oct 2016; 1 male specimen deposited at UFMG AC, cave PDI_0052, 19°53'59.82"S, 43°28'7.34"W Datum WGS84, col Oliveira MP, 17 Oct 2016; 3 female and 1 male specimens deposited at UFMG AC, cave PDI_0065, 19°53'58.12"S, 43°28'14.68"W Datum WGS84, col Oliveira MP, 12 Oct 2016; 1 female specimen deposited at UFMG AC, cave PDI_0059, 19°54'0.75"S, 43°28'9.91"W Datum WGS84, col Oliveira MP, 19 Oct 2016; 1 male specimen deposited at UFMG AC, cave PDI_0084, 19°53'52.83"S, 43°28'11.95"W Datum WGS84, col Oliveira

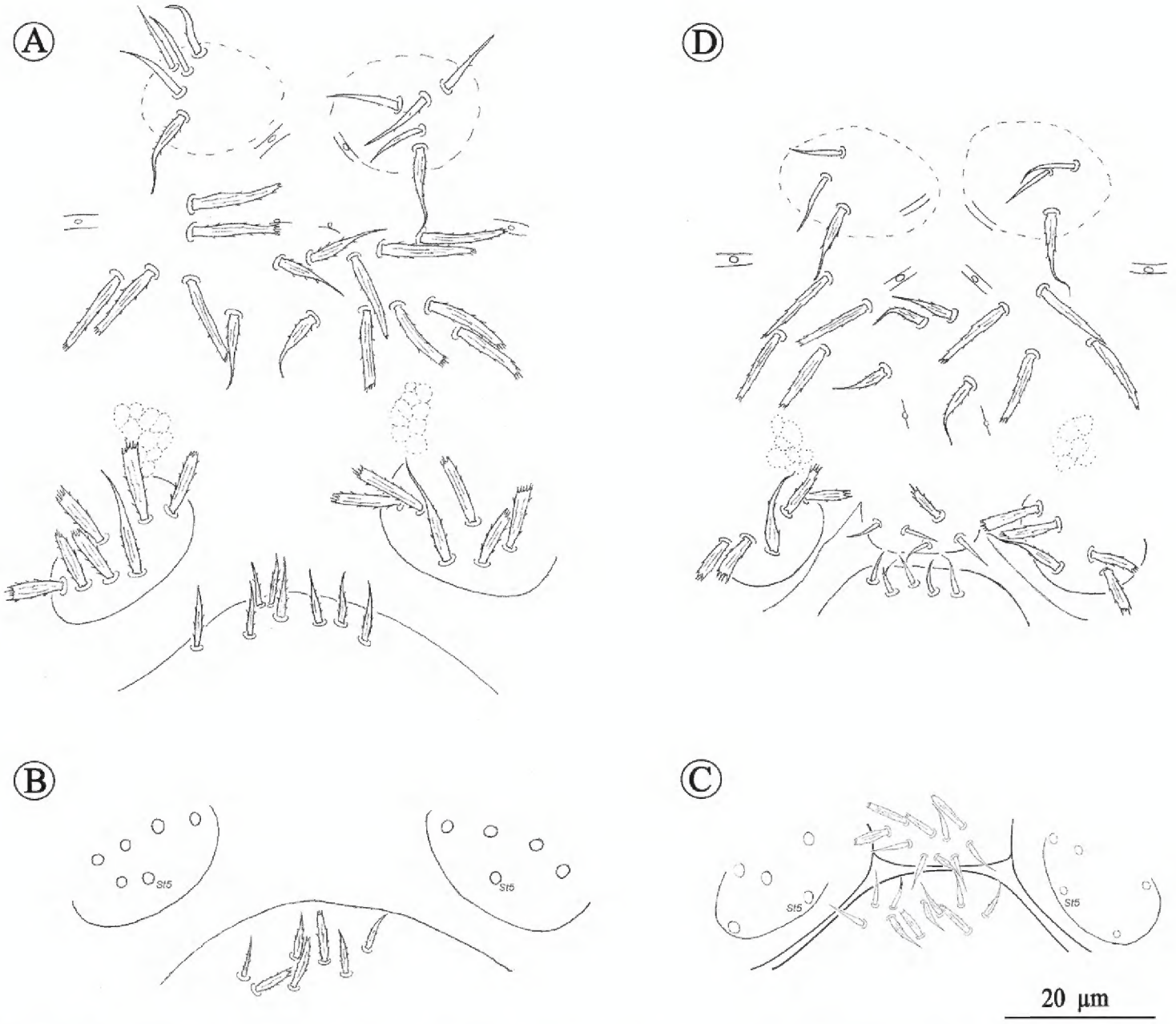

Figure 8. *Neocarus simmonsi* sp. nov., view of sternitogenital region; View of female sternitogenital region (**A**) and view of variotion on genital region (**B**); View of male sternitogenital region (**C**) and view of variation on pregetial and genital region (**D**).

MP, 24 Nov 2015, 1 female specimen deposited at UFMG AC, cave MDIR_0015/16, 19°53'28.88"S, 43°27'40.17"W Datum WGS84, col Oliveira MP, 10 Oct 2016.

Etymology. The specific name is in honor of George C. Simmons due his contributions to studies on caves genesis and mineralogy. Simmons produced seminal papers in iron ore caves and karst in Brazil and one of his important research projects was conducted in cave MDIR_0020 (Simmons cave), near the type locality of the new *Neocarus* species.

Comparative notes. *Neocarus simmonsi* differs from *N. potiguar* Bernardi, Zacarias & Ferreira, 2012, *N. proteus* Bernardi, Klompen, Zacarias & Ferreira, 2013, and *N. platensis* (Silvestri, 1905) from Brazil by the absence of pregenital setae in the female. It differs from *N. coronatus* Araújo & Feres, 2018 by the presence of 6 (vs. 4) foliate setae on the palp tarsus; from *N. potiguar*, *N. caipora* Bernardi, Klompen & Ferreira, 2014, *N. platensis* (from Argentina and Uruguay) and *N. misiones* Vázquez, Bernardi & Klompen, 2020 by the absence (vs. presence) of *p*-type setae on the palp genu (*N. pro-*

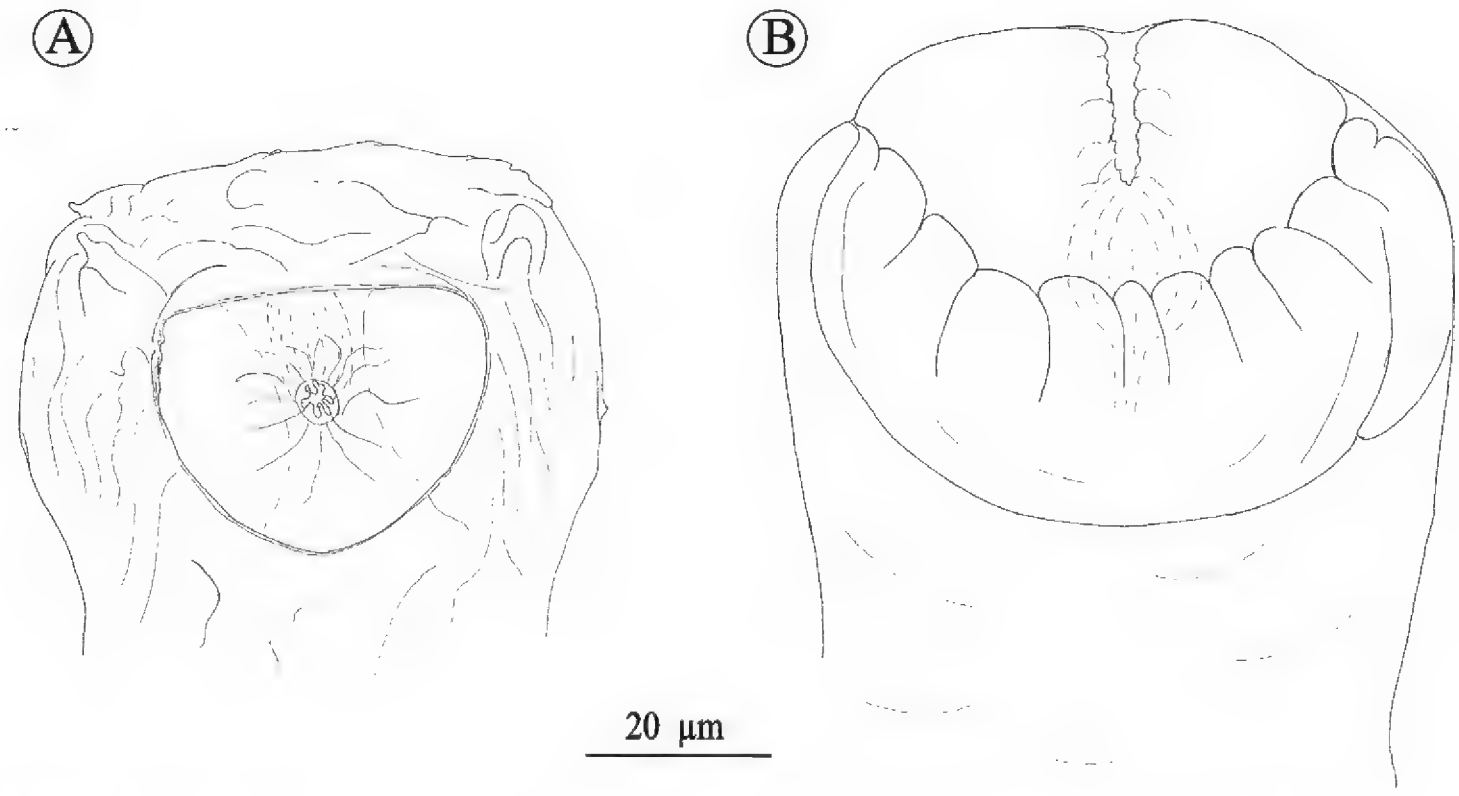

Figure 9. *Neocarus simmonsi* sp. nov., view of invaginated ovipositor (**A**) and evaginadted (**B**).

teus and *N. spelaion* Bernardi, 2018 are somewhat intermediate as they carry small numbers (1–7) of *p*-type setae on the palp genu), and from *N. spelaion* by the uniformity (vs. variability) in shape of the pregenital and genital setae in the male. *N. simmonsi* differs from *N. entrerios* Vázquez, Bernardi & Klompen, 2020 and *N. coronatus* by the absence (vs presence) of sexual differentiation in the setae on the prodorsal shield.

The ovipositor is a unique structure and its characteristic is useful to differentiate Opilioacarida species. *Neocarus simmonsi* presents an ovipositor cylindrical with a rounded tip similar only to *N. potiguar*, but differentiates from the species with terminal lobes, such as *N. misiones* (three small and very distinct roundish terminal lobe), *N. entrerios* (with a distinct pair of papillate hooks), *N. spelaion* (with a rounded and distinct lobes) and *N. proteus* (two rounded structures plus three membranes at tip. *Neocarus coronatus* and *N. caipora* differs from all South American species due the presence of setae on ovipositor. *Neocarus platensis* and *N. ojastii* have a poorly described ovipositor structure.

Life history remarks

Development and morphological abnormalities

The ability to regenerate appendages can be a great advantage for species of Opilioacarida since these organisms easily lose their appendages during different stages of development or even in adulthood (Coineau and Legendre 1975; Klompen 2000; Bernardi et al. 2013a). As described in previous works (Coineau and Legendre 1975; Klompen 2000; Bernardi et al. 2013a), regeneration has been observed in young specimens of

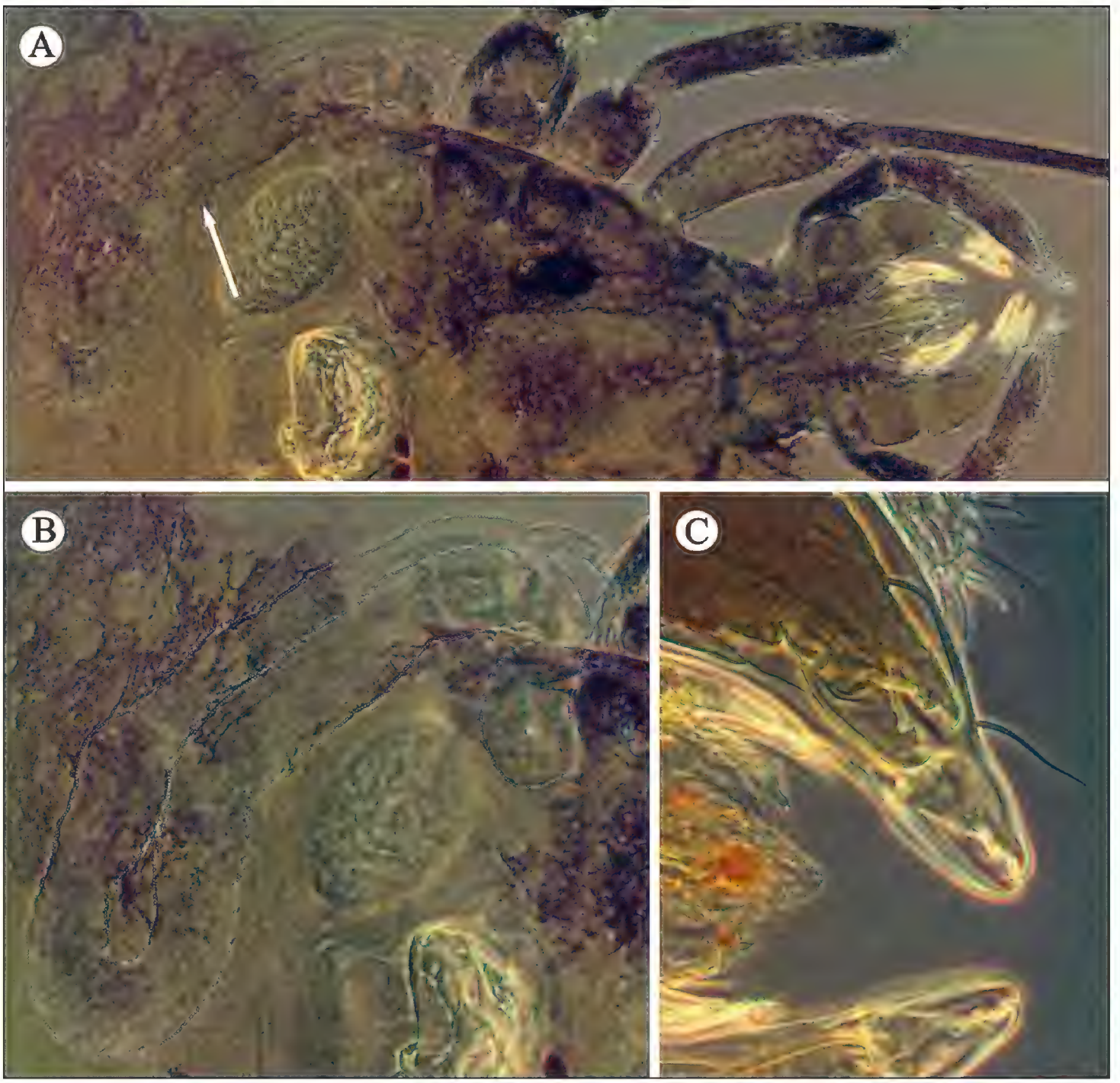

Figure 10. *Neocarus simmonsi* sp. nov., view of regeneration process on leg (arrowed) in adult female (**A, B**); details of the molting process on chelicera.

N. simmonsi, namely deutonymphs and tritonymphs. Here, we observed for the first time this replacement of appendages in adults. The specimen, a female, was collected while changing its integument, which made it possible to observe the old integument and the new integument formed below, with leg IV in regeneration (Fig. 10). Integumentary molting and continuous growth in Opilioacarida, even in adulthood, can be a physiological advantage for species of this group because they have a fragile integument, a possible autotomy behaviour and these mites can easily lose appendages.

It is not possible to say how many molts an adult can experience even after reaching sexual maturity, but the size of a male and female collected during field study suggests that growth may continue, and thus there can be more than one molt in adulthood. This is based on the observation that collected adult specimens exhibited the molting process, with two specimens (1 male and 1 female) having an old integument (exuvia), which was covering the new one. The new integument presented some

Table 1. Comparative setal patterns and shape for the pregenital and genital region, ovipositor and palp of *Neocarus* adults.

OCCURENCE	SPECIES/ SUBSPECIES	FEMALE		MALE		Palp	
		Pregenital region	Genital / Eugenital region	Pregenital region	Genital region	*ch*-type	*d*-type
		No. and type of setae	No. and type of setae	No. and type of setae	No. and type of setae		
North America							
USA	*Neocarus texanus*	2 st/r	nude	4–6 st/r	8–9 sh	10–14(21*)	5
Mexico	*Neocarus nohbecanus*	nude	nude	4–5 st/r	5–7st/r	17–19	4
Mexico	*Neocarus siankaanensis*	nude	nude	2 st/r	4 st/r	14–15	5
Mexico	*Neocarus bajacalifornicus bajacalifornicus*	2 st/r	nude	5–8(13a) st/r	7–8(11a) st/r	14–18 (21a)	5
Mexico	*Neocarus bajacalifornicus chamelaensis*	2–3 st/r	nude	4–5 st/r	4–6 st/r	16	5
Mexico	*Neocarus calakmulensis*	2–3 st/r	nude	2–6 st/r	3–8 st/r	17	5
Mexico	*Neocarus veracruzensis*	2 st/r	nude	6–8 st/r, 0–1 s	6–8 st/r	13	5
Mexico	*Neocarus comalensis*	5–7 st/r	3 st/r			14–18	5
Mexico	*Neocarus chactemalensis*	nude	nude	4–6 st/r	4–6 st/r	11–13	4
Central America							
Nicaragua	*Neocarus nicaraguensis*	2–5 st/r	nude	2–7 st/r	3–6 st/r	18–22	5 or 6
Cuba	*Neocarus orghidani*	nude	nude	4–5 st/r	5–7 st/r	20–24	4
Belize	*Neocarus belizensis*	nude	nude	2–3 st/r	4–5 st/r	17–21	5 or 6
South America							
Venezuela	*Neocarus ojastii***	nude	nude	6–9?	13 st/r	–	–
Brazil	*Neocarus proteus*	2–5 st/r	4–6 wb	2–5 st/r	3–5 sh	12 or 13	5 or 6
Brazil	*Neocarus potiguar*	1 tp/r	4–8 sh	5 st/r	7–10 st/r	25–27	5 or 6
Brazil	*Neocarus coronatus*	nude	6 tp/b	1–7 st/r	5–15 tp/r	18–25	4
Brazil	*Neocarus caipora*	nude	8–12 sh	4–8 st/r	5–8 tp/r	15–16	6
Brazil	*Neocarus spelaion*	nude	10–12 sh	9–12 sh or tp/r	7–11 sh and/ or tp/r	14–18	5 or 6
Brazil	*Neocarus simmonsi*	nude	6–12 st/b or tp/b	4–10 sh or tp/r or st/r	4–10 sh or tp/r or st/r	15–17	6
Brazil/Argentina/Uruguay	*Neocarus platensis*	0–2 st/r	6–9 sh	6–10 st/r	5–10 sh	14	5 or 6
Argentina	*Neocarus misiones*	nude	12–13 tp/b	7–9 st/r and/ or tp/b	12–13 tp/b	15	6
Argentina	*Neocarus entrerios*	nude	6–12 sh	6–10 st/r	8–10 tp/r	20	6

* number of setae in super adults. **The description of *Neocarus ojastii* does not provide sufficient detail for an adequate comparison. Setae type: sh: smooth setae; st/r – stout and ribbed setae; tp – tapering; tp/b: tapering and barbed setae; wb: weakly barbed setae.

characters already 9 to 18% larger than in any of the other adults collected. Based on the measurements of body setae, Bernardi et al. (2013a) suggested that there is a continuous increase in adult body size after molts. Vazquez and Klompen (2002) observed a greater number of setae in super-adults, but this morphological change was not observed in the specimens studied here.

Another interesting observation made on an adult male of *N. simmonsi* was the presence of morphological abnormalities of the palp, with the reduction and reorganization of setae as well as changes in the shape of the structure itself (Fig. 11). Small abnormalities in body setae (Araújo et al. 2018a) or the duplication of pores (Bernardi and Borges-Filho 2018) has been observed in other species. Here, for the first time, abnormalities are seen in complete structures, such as the palp. The potential behavioral consequences for individuals remain unclear.

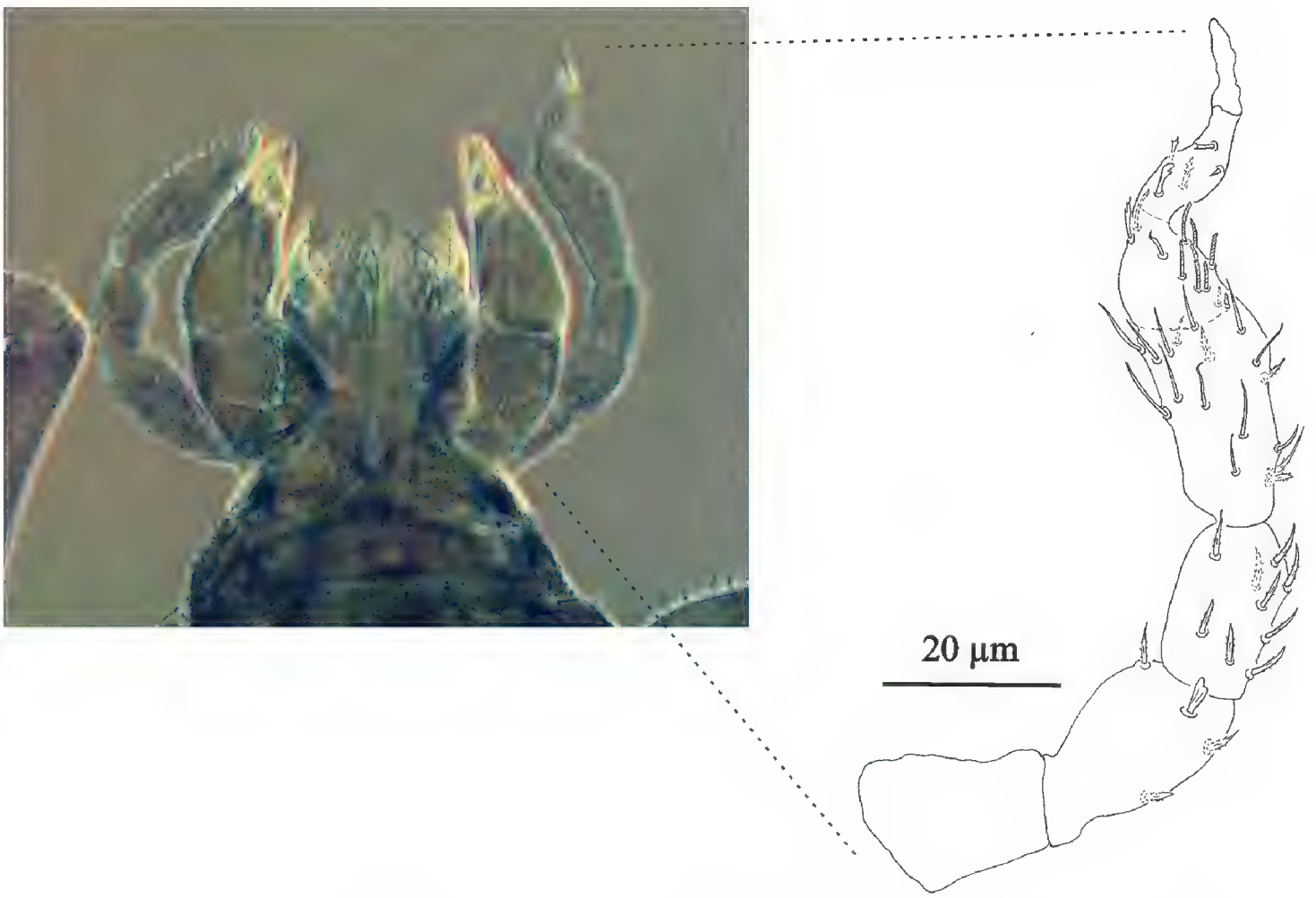

Figure 11. *Neocarus simmonsi* sp. nov., detail view of the morphological abnormality on palp of male.

Gut contents

The gut contents of specimens of *N. simmonsi* includes unidentified plant fragments, arthropod remains, pollen, and fungal hyphae (Fig. 12). The observation of plant fragments and pollen in the stomach contents of specimens collected in the deepest areas of caves (more than 40 m from the entrance to aphotic zones in some caves) suggests that these organisms feed on decomposing matter from the epigean environment that is transported by water action. Three specimens of *N. simmonsi* had also specimens of mites, possibly of the family Tarsonemidae and Oribatida. This feed plasticity can assist in the colonization of a great diversity of habitats, including caves, where resources generally tend to be scarce.

In addition to these materials, the stomach contents of three specimens evaluated under microscopy possessed remains of integuments (exuvia) of Opilioacarida (Fig. 12). When reviewing the stomach contents of other specimens of other species present in the collect of Universidade Federal de Lavras (ISLA), we also observed materials of this nature in *Caribeacarus brasiliensis* (4 individuals), *N. caipora* (3 individuals), *N. proteus* (4 individuals) and other undescribed species of Opilioacarida (8 individuals).

During the observation of *N. proteus* kept laboratory condition in 2012, for about 4 months, also allowed documenting specimens feeding on their own exuvia after molting (2 observations) and even exuvia of other dead individuals (2 observations). Such

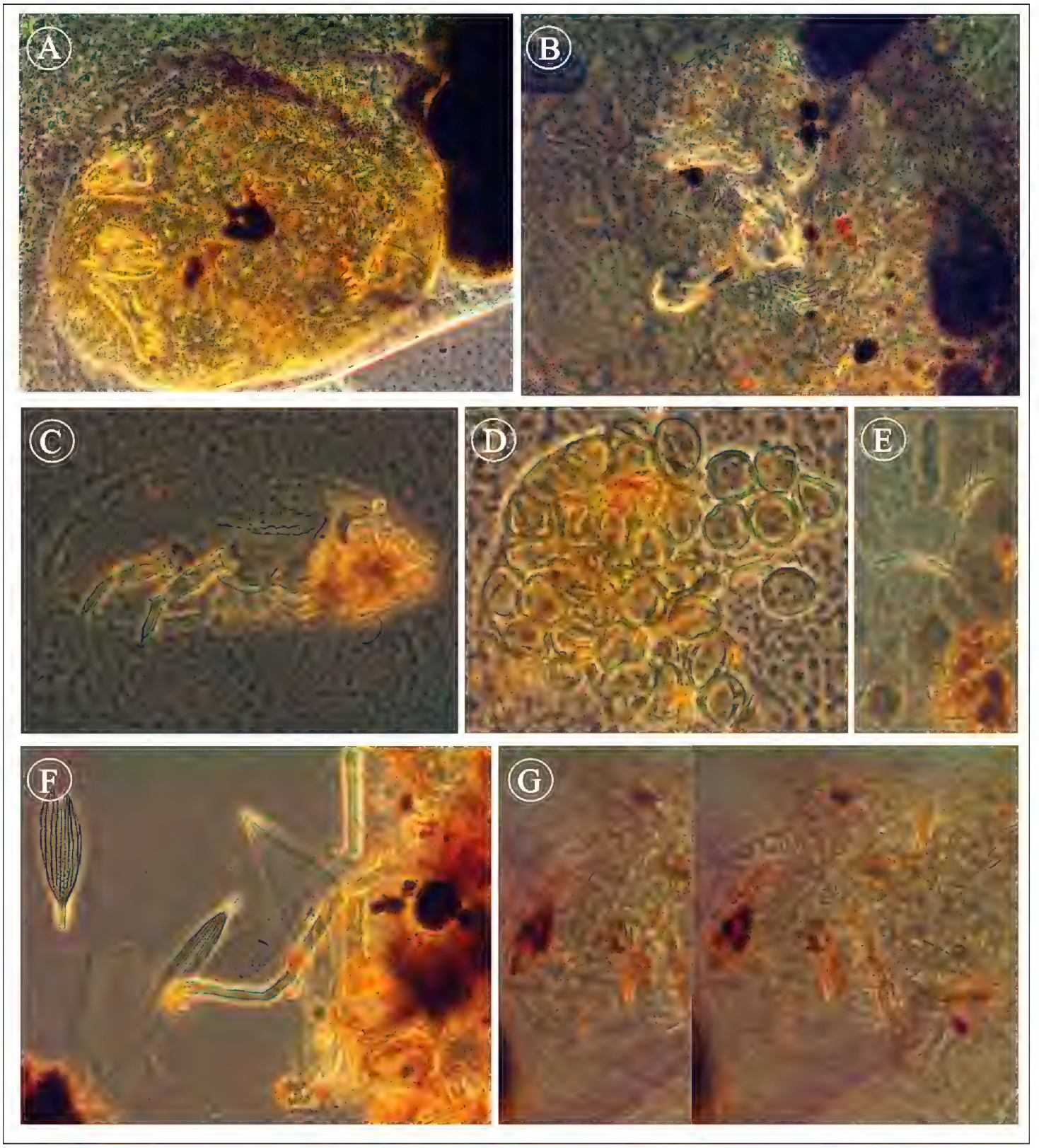

Figure 12. Example images of the gut content of individuals *Neocarus simmonsi* sp. nov., **A, B** exuvia of Opilioacarida, **C** mite (Astigmatina), **D** pollen, **E** probably a bothridial seta (oribatida), **F** plants, fungi and arthropods parts, **G** mite exuvia, probably an Endeostigmata.

laboratory data, associated with the observations of stomach contents of specimens of *N. simmonsi* in the present study and the absence of records of cannibalism among Opilioacarida bred and maintained in the laboratory (Vázquez and Palacios-Vargas 1989; Klompen 2000; personal observations), allows us to infer that these species feed on their own exuvia. This behavior is not unique among arachnids, but is apparently rare. Some observations made by Dondale (1965) suggest that spiders feed on exuvia because they contain a set of proteins that can be an important source of nutrients for

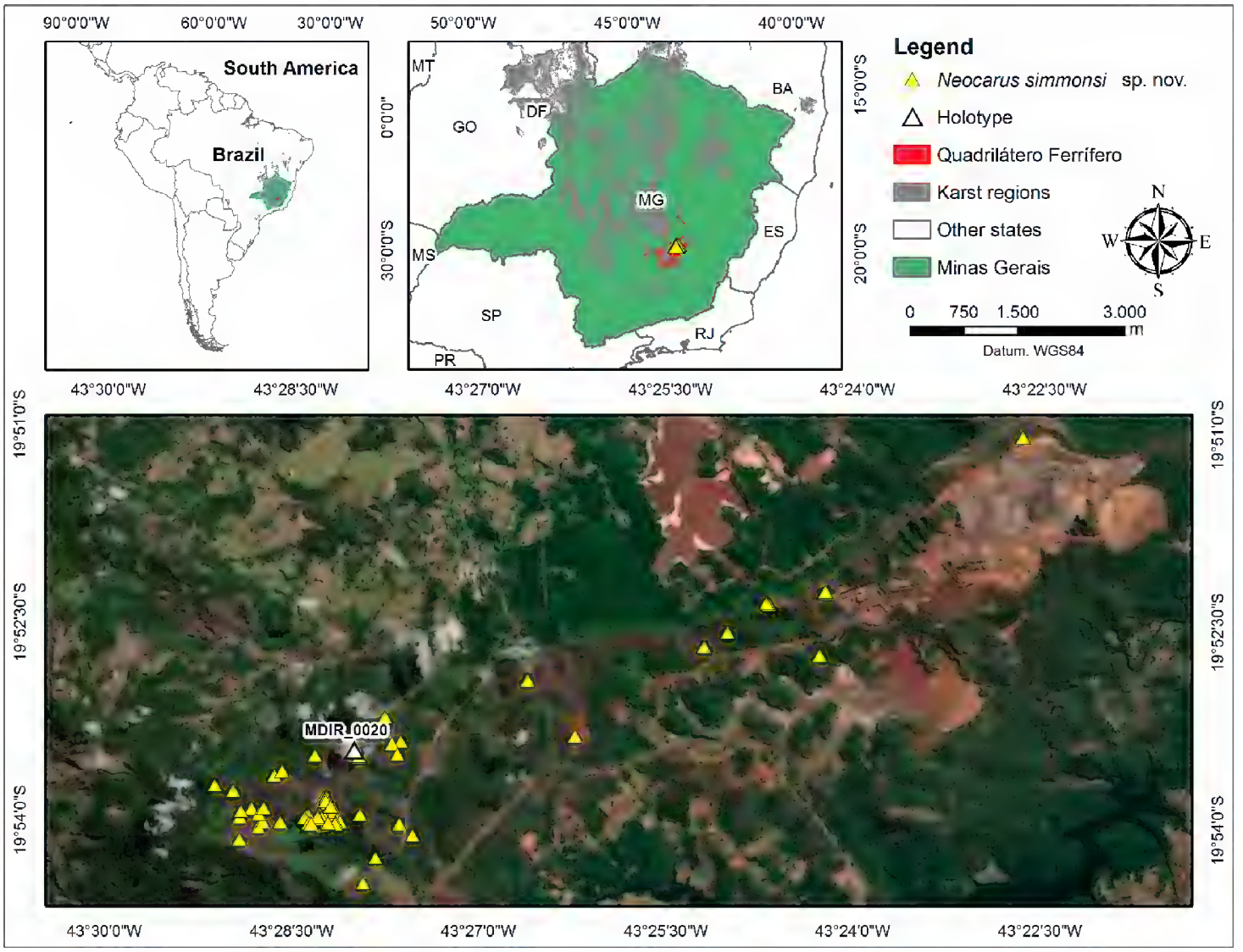

Figure 13. Distribution of *Neocarus simmonsi* sp. nov..

individuals after going through the stress of molting, and the period without energy intake. This is an interesting behavior, because it allows Opilioacarida to reabsorb energy present in the tissue or liquid, discarded in the process of ecdysis. This behavior can be an important strategy in an environment with scarce resources, such as some caves.

Distribution, habitat, and seasonality

Neocarus simmonsi does not have morphological characteristics arising from isolation in subterranean environments and was found both in caves and the epigean environment. The only species whose morphology has been modified to the point of being considered troglobitic are *Siamacarus dalgeri* and *S. withi*, described by Leclerc (1989) from caves in Thailand. As adaptations to the subterranean realm, these species lack eyes, possess elongated appendages, have a pale coloration, and even modified sensory setae. Similar traits have not been found in cave species in Brazil thus far.

The distribution of *N. simmonsi* extends across the entire Serra do Tamanduá and Dois Irmãos (municipalities of São Gonçalo do Rio Abaixo and Barão de Cocais) in a strip of at least 12 km. However, the effective distribution of the species is expected to be much greater, considering that it is a common species in the sampled area and that it is not restricted to subterranean environments (caves) (Figs 13, 14).

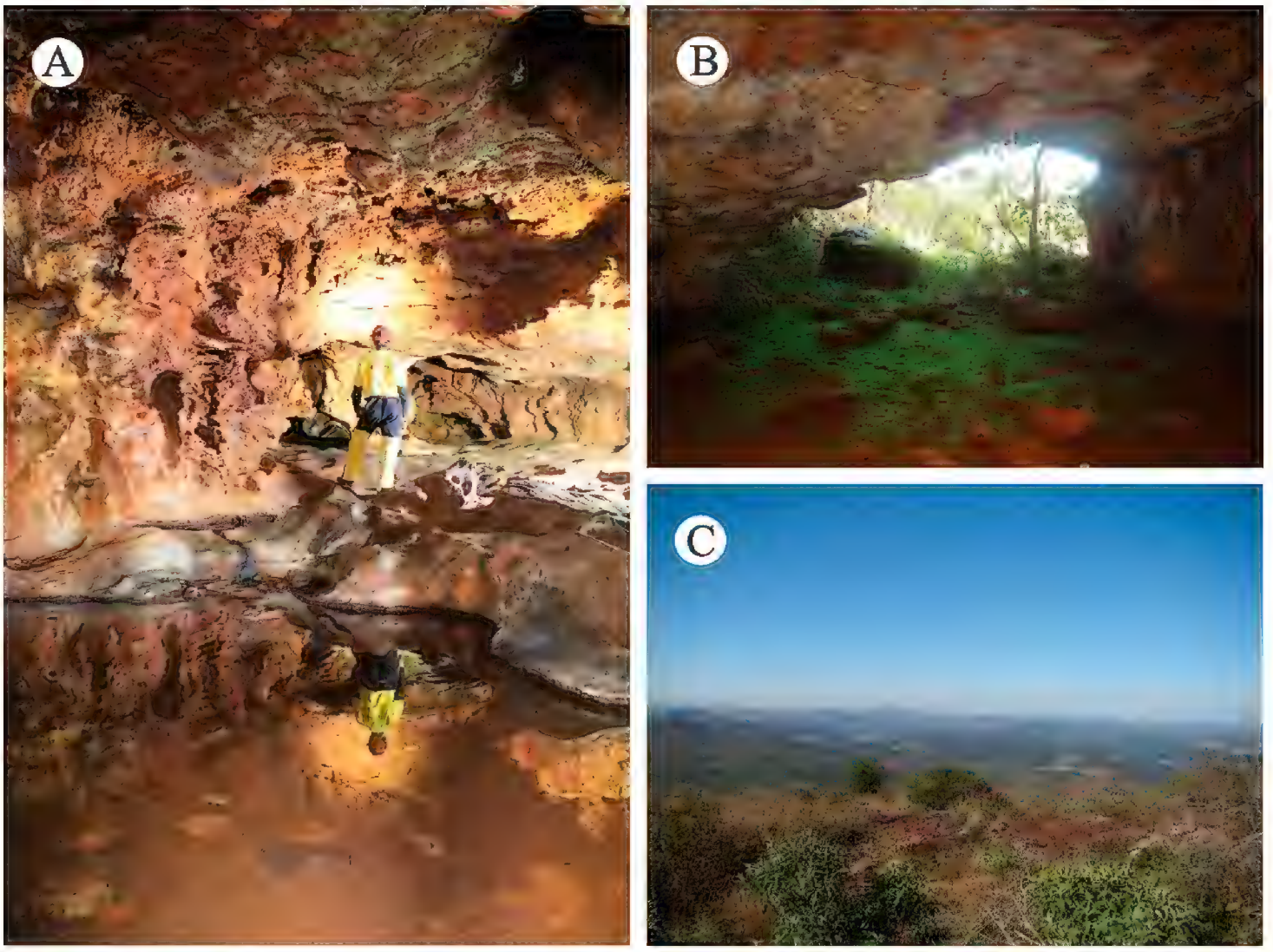

Figure 14. General aspects of Simmons' cave (MDR_0020), (**A, B**) and epigean environmental (**C**), habitat of *Neocarus simmonsi* sp. nov.

The study area is located northeast of the Quadrilátero Ferrífero (Iron Quadrangle), in an area of Atlantic Forest near the transition zone with the Cerrado biome (Brazilian savanna). The vegetation cover consists of forest formations and "campos rupestres" (rupestrian fields) at higher altitudes and shallow soils rich in metals (e.g. iron). Since it is a geomorphologically diversified area and located close to the transition between two biomes, there is a great diversity of flora and fauna. The region, therefore, is considered a priority area for conservation in the state of Minas Gerais with "extreme biological importance" due to the high floristic and faunal richness and the presence of several endemic and endangered species (Drummond et al. 2005). There are a few hundred registered caves in the study area including the largest caves in the Quadrilátero Ferrífero.

The type locality, Simmons' cave, was mapped in 1960 and is one of the few examples of caves formed by dissolution in the region. The cave is located at approximately 1140 meters above sea level and has only one entrance, aphotic zones, different compartments (halls), perennial lakes, and 146 meters of horizontal projection. With regard to organic resources, litter deposits were observed in the entrance area, along with guano of carnivorous and hematophagous bats. Lichens and fungi were observed on the walls and floor near the entrance to the cavity.

Neocarus simmonsi is an abundant species in caves in the region, having been found in 73 of the 109 studied caves. The abundance of specimens was significantly higher in

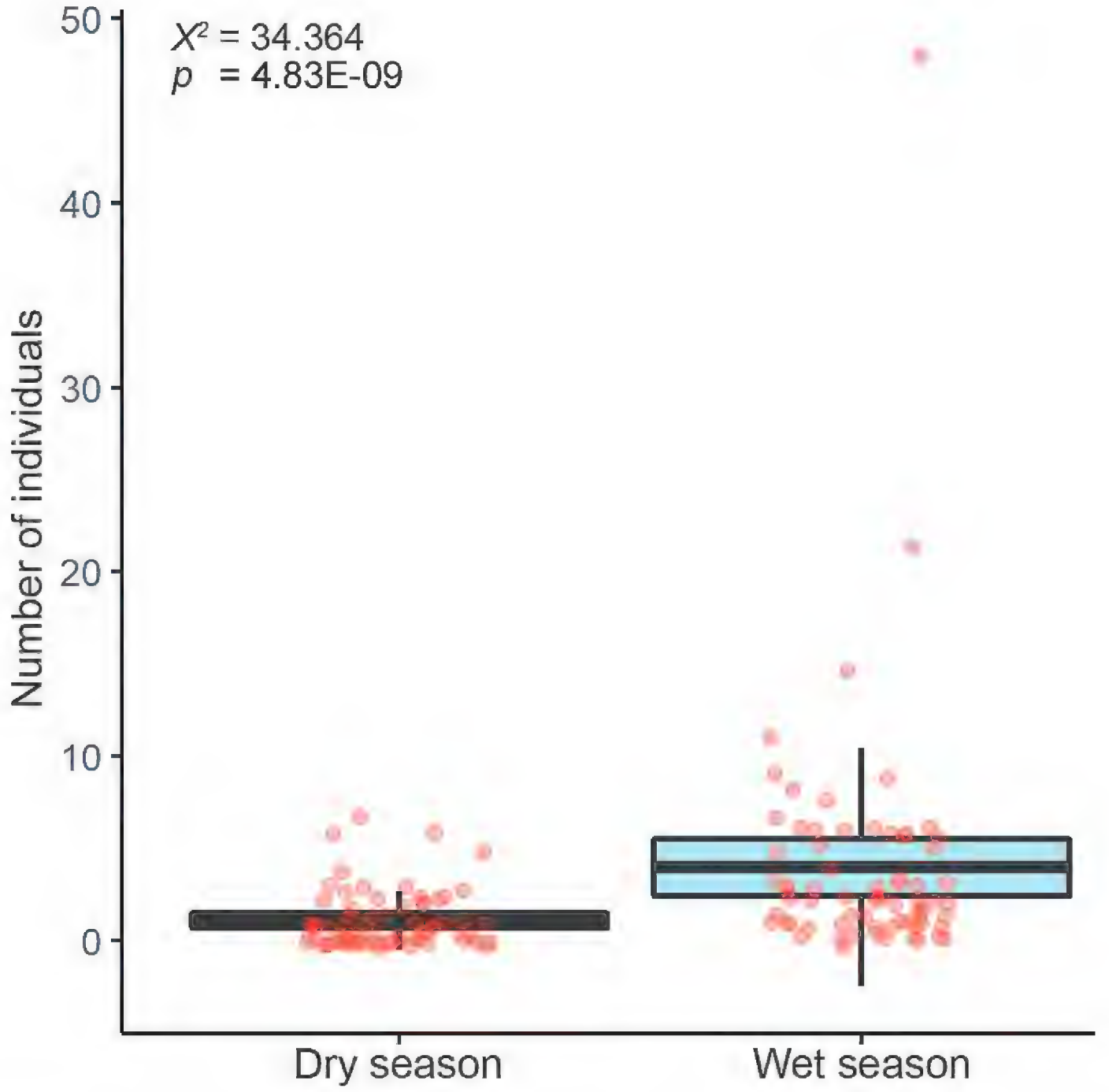

Figure 15. Average sampled individuals of *Neocarus simmonsi* sp. nov. per cave according to seasonality. The abundance values are significantly different between the two seasons according to the Generalized linear model analysis (z = 5.853; *p-value* = 4.83e^{-9}) In the boxplots, the turquoise areas refer to the inter-quartile range around the observed median (central black line) and vertical bars represent the maximum-minimum range (excluding outliers).

the wet season (estimated $\beta \pm$ S.E. = 1.29328 $\pm$ 0.22096; z = 5.853; *p-value* = 4.83e^{-9}), ranging from few to dozens of individuals as the case of BRU_0012 cave (n = 48 during the wet season and n = 0 during the dry season), BRU_0023 cave (n = 21 during the wet season and n = 1 during the dry season), and BRU_0002 cave (n = 15 in the wet season and n = 1 during the dry season). This great oscillation may be explained by the fact that the number of individuals observed increases during the rainy season, or else it may be due to the migratory behavior of the species (Fig. 15). Even in caves, where the environment is generally stabler than at the surface, seasonality can still influence the general climate (Bento et al. 2016; Mammola and Isaia 2018), and decrease the availability of water and food (Simon et al. 2007; Silva et al. 2011; Souza-Silva et al. 2012). Nevertheless, further investigations are needed to verify the generality of this pattern.

In some caves individuals of *N. simmonsi* were found aggregated in groups of up to eight and ranging from protonymph stages to adult males and females. The species' behavior is similar to that observed for *N. caipora* and *Caribeacarus brasiliensis* (Bernardi et al. 2014). As suggested by Vázquez et al. (2018), this gregarious behavior can increase the survival rate, especially for young individuals. *Neocarus simmonsi* was significantly more abundandant during the wet season (Fig. 15), when there is greater rainfall in the region (above 1000 mm). Besides climate, another factor that may determine variations in the abundance of this species is the availability of organic resources. Organic matter is imported into caves by biological agents (e.g. bats), wind or rain (Simon et al. 2007; Silva et al. 2011; Souza-Silva et al. 2012), with this transport being generally greater during the wet season.

Final remarks

The number of described species of Opilioacarida from collections in karst areas and caves in Brazil is increasing (Bernardi et al. 2013b, c, 2014; Araújo et al. 2018a; Bernardi and Borges-Filho 2018). This is due to the intensification of sampling efforts in this habitat as the result of environmental licensing processes. Environmental licensing requires speleological studies to be carried out in karst areas or other sites that potentially bear caves in Brazil among them the inventory of cave fauna (BRASIL, 6.640, IN MMA 2017). This legal requirement has lead to the discovery and description of several new species in the country, in addition to the acquisition of information on the range and biology of various species.

Brazil stands out for its diversity of described species of Opiliacarida (11 spp.) and its great potential for new records in view of its territorial extension and the large concentration of caves in different lithologies and biomes (Lewinsohn and Prado 2005; Oliveira et al. 2016; CECAV 2019). Thus, an increase in the number of new species is expected with intensification of sampling effort in different ecosystems and greater investment in taxonomic research and training of new specialists.

Mites and other soil invertebrates generally exhibit seasonal fluctuations, with their richness and abundance being determined by environmental factors such as precipitation and temperature (Badejo 1990). Although caves are confined systems and have greater environmental stability than the surrounding epigeal environment (Culver 1982; Freitas and Littlejohn 1987), the cave microclimate can also influence the structure and composition of fauna, with fluctuations in populations and communities throughout the year. Such variation is often a response to variation in the external climate (Ferreira et al. 2015; Bento et al. 2016), as indirectly observed for *N. simmonsi*.

Opilioacarida is a group of species with interesting feeding habits. The material ingested by them is composed of large fragments of solid material of vegetable, animal and/or microbiological origin (van der Hammen 1966; Walter and Proctor 1998; Klompen 2000; Araújo et al. 2018a; Bernardi and Borges-Filho 2018). Although small invertebrates (especially mites) have been observed in the stomach contents of *N. simmonsi*, it is still not possible to say whether species in this group are predators or scavengers. For example, our observations of the stomach contents of *N. simmonsi*

would suggest that these organisms are one of the few known "*scavengers*" of Parasitiformes, but this remains an anecdotal observation.

Finally, the order Opilioacarida always occupies a prominent position when referring to the study of development among mites. The development of these taxa comprises an embryonic phase (prelarva), subsequent larva, protonymphs, deutonymphs, tritonymphs and adults, but its growth can continue beyond the adult phase. The regeneration of appendages in adult individuals, however, is a peculiarity known only in this group among Parasitiformes and is rarely observed among a few Acariformes and arachnids in general (Michener 1946; Imamura 1952; Furumizo and George 1976; Bernardi et al. 2013a). Growth in adulthood can result in individuals with larger bodies and morphological structures and a greater number of setae in some areas of the body (Vázquez and Klompen 2002; Bernardi et al. 2013a). In the present study, we observed that growth and regenerative ability occur in all life stages of Opilioacaridae, which may eventually increase their survival rate.

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